Diuresis among Last Instar Nymphs of Periplaneta americana L. when Treated with Custard Apple Seed (Annona squamosa L.) Extract

AUTHOR(S):

QADRI, SYED S.H.; HASAN, S.B.

CITATION:

QADRI, SYED S.H. ...[et al]. Diuresis among Last Instar Nymphs of Periplaneta americana L. when Treated with Custard Apple Seed (Annona squamosa L.) Extract. 防虫科学 1975, 40(1): 1-5

ISSUE DATE:

1975-02-28

URL:

http://hdl.handle.net/2433/158871

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Diuresis among Last Instar Nymphs of *Periplaneta americana* L. when Treated with Custard Apple Seed (*Annona squamosa* L.) Extract. Syed S.H. Qadri & S.B. Hasan (Department of Zoology, Post-Graduate Centre, Warangal, A.P., India) Received September 20, 1974. *Botyu-Kagaku* 40, 1, 1975.

1. Custard apple の種子抽出物によるワモンゴキブリ終令幼虫の利尿 Syed S.H. Qadri and S.B. Hasan (Department of Zoology, Post-Graduate Centre, Warangal, A.P., India) 49. 9. 20 受理

Custard apple (*Annona squamosa* L.) の種子の抽出物をワモンゴキブリ *Periplaneta americana* L. 最終令幼虫に経皮的に施用すると，直腸に尿が多くたまる．その抽出物を有機塩素剤や有機燐剤に加用すると，共力作用が認められ，利尿作用はそれぞれ単独で施用した場合よりも一層いちじるしい．抽出物単独，あるいはこれらの殺虫剤との混用による体内血液量の減少は，体の水分平衡の臨界を乱すことになり，体の乾燥を促進することになる．持続性のない植物成分を，急性致死的な麻痺剤として用いるのではなく，昆虫の排泄に異常をおこさせる薬剤として応用できるかも知れない．

Seeds and roots of custard apple (*Annona squamosa* Linn.) contain an insecticidal material which, when concentrated with ether appears to be about as potent against several insect species as rotenone[6]. Further, an insecticidal material is also precipitated out with petroleum ether at 0°C from the custard apple seed extract concentrated with ether[3]. Ether extract showed repellency when rice weevil (*Sitophilus oryzae* L.) and flour beetle (*Tribolium castaneum* Hbst.) were subjected to the treated wheats[9]. Synergism between chlorinated hydrocarbons like γ-HCH and organophosphorus insecticides with custard apple seed extract was also observed when tested against *S. oryzae* as surface contact[10]. But, so far no attempts have been made to elucidate the mechanism of action of custard apple seed extract against insect pest as toxicant and synergist for γ-HCH, malathion, fenitrothion and parathion. Therefore, in the present studies an attempt has been made to explore the possiblities of its action against house-hold insect like *Periplaneta americana* L.

Materials and Methods

Insecticides:

The insecticides and synergists used were: Lindane (γ-hexachlorocyclohexane), malathion (S-[1,2-di-(ethoxycarbonyl)-ethyl] dimethyl phosphorothiolothionate), fenitrothion (dimethyl 3-methyl-4-nitrophenyl phosphorothionate), parathion (diethyl 4-nitrophenyl phosphorothionate)

Custard apple (*Annona squamosa* Linn.): Custard apple is distributed throughout India and belongs to the genus Annona and family Annonaceae. The plant source is extensively used as detergent[4], and as a curative of diseases[1]. Seeds, leaves and immature dried fruits are used as an inseticide for bedbugs, head and body lice[6]. A poultice of the leaves with salt is applied to boils, ulcers, maggot infested sores, malignant tumours and to extract guinea worms[1,2].

Extract of A. squamosa: *Ether extract*: The seeds were obtained by passing through 140 mesh sieve. The powdered material (1,000 gm) was extracted in the cold with ether (b.p. 33°C). The yield of the residue, on removal of the solvent was 60 gm (6.0 per cent w/w on the weight of seeds). From this residue with petroleum ether an insecticidal material was precipitated out at 0°C as described by Feinstein[3]. For use as toxicant and synergist the residues were dissolved in acetone to yield solutions of suitable concentrations.

Toxicological Method: To determine the diuretic activity of the extract, nymphs of *P. americana* of last instar were drawn from laboratory cultures and used in the experiments. The insecticides

and solvent extract of custard apple seed were dissolved in acetone and applied topically on the third ventral abdominal segment with Agla all-glass micrometer syringe at the rate of 0.005 ml/gm body weight of the cockroach. There were 5 insects in each batch and 3 such batches were used in each assay. All mortality counts were made after exposing the insect at 28±2°C for 18 hours.

Method for diuretic activity of custard apple seed extract: To collect the haemolymph cockroaches were collected 18 hours after the exposure at 28±2°C by the method of Sternburg and Corrigen[14]. In this method the anus of cockroaches were sealed soon after the treatment both in control and treatment lots with melted paraffin wax. 18 hours after the treatment when the insects started getting prostrated and paralized their blood was collected by sealing their mouth with melted paraffin wax and cutting their legs, and antennae. The collected blood was immediately kept in a beaker holding ice. Then the amounts of haemolymphs in both treated and untreated cockroaches were estimated. At least three batches of cockroaches each consisting of 3 individuals in each assay were used to collect the total haemolymph, and the average amounts of haemolymph/batch of cockroaches were calculated and are shown in Table 3.

After haemolymph had been taken from the treated and untreated insects, they were dissected so that the quantity of the rectal fluid in the rectum could be estimated from a measurement of its area made with an ocular micrometer. A minimum of three replicate dissections were made and the average values of rectal fluid obtained are shown (in Tables 1 & 2) for individual and combination of insecticide and custard apple seed extract.

Results

Toxicity against last instar nymphs of. P. americana: Relative toxicity of the solvent seed extract of the custard apple and storage insecticides was studied on the basis of the mortality recorded among the insects administered measured doses topically as described earlier. The data obtained are shown in Table 1.

The data in Table 1 suggest that custard apple seed extract was less toxic to cockroach nymphs as compared to storage insecticides used. In order of relative toxicity parathion was followed by the·fenitrothion, γ-HCH, malathion and custard apple seed extract.

Joint action with γ-hexachlorocyclohexane, malathion, fenitrothion and parathion against last instar nymphs of P. americana: In preliminary experiments, the ratio of plant synergist to chlorinated hydrocarbon and organophosphorus compounds was determined to the optimum value. The ratios were as follows: 6 parts, 10 parts, 30 parts and 10 parts of custard apple seed extract to 1 part of γ-HCH, malathion, fenitorthion and parathion respectively against cockroach nymphs. The synergistic effects of solvent extract of custard apple seed with chlorinated hydrocarbon and organophosphorus compounds was observed by plotting the regression lines for the compounds

Table 1. Insecticides tested and found to cause the accumulation of the rectal fluid (diuresis) among the last instar male nymphs of *P. americana* 18 hours after the topical application.

Insecticide	LD_{50}* μg/g body weight	Relative toxicity	Diuresis** (in microns)
Custard apple	1,500.00	0.0001	15.16
γ-HCH	1.00	0.25	15.16
Malathion	12.50	0.02	4.13
Fenitrothion	0.625	0.04	6.89
Parathion	0.25	1.00	1.38

* Read from the regression lines plotted between Probit kill/Log concentration.

** Based on the measurement of the bulging of the recta due to the accumulation of the rectal fluid by ocular micrometer.

Table 2. Analysis of synergistic of custard apple (Syn.) assuming joint action with γ-HCH, Malathion, Fenitrothion and Parathion in various ratios and found to cause accumulation of the rectal fluid (Diuresis) among the last instar male nymphs of *P. americana* 18 hours after the topical application.

Compound	Ratio	LD_{50}* μg/g body wt.		Wadley's Synergism ratio	Diuresis** (in microns)
		Alone	Mixture		
Custard apple (Syn.)	—	1,500.00			
γ-HCH	1:6	1.00	0.16	6.25	28.94
Malathion	1:10	12.50	2.00	6.25	19.30
Fenitrothion	1:30	0.25	0.10	2.50	35.83
Parathion	1:10	0.625	0.04	15.62	28.94

* Read from the regression lines plotted between Probit kill/Log concentration.
** Based on the measurement of the bulging of the recta due to the accumulation of the rectal fluid by ocular micrometer.

alone and the synergist, and the values for LD_{50} were read from these lines and are shown in Table 2. Synergism of the plant material was assumed since the regression lines were parallel, and in all cases lines for joint action were displaced to the left.

From Table 2 it will be seen that all the values for mixture are smaller than alone. Wadley's synergism ratio[15] also indicates clear cut evidence of synergism.

Diuresis in the last instar nymphs of P. americana: Diuresis in the last instar nymphs of cockroach was studied on the basis of the topical administration of the measured doses of the individual and mixture of insecticide and custard apple seed extract separately on the 3rd abdominal sternum as described earlier and the data obtained are shown in Tables 1 & 2.

The data in Table 1 suggest that custard apple seed extract was more or less equal in effect to γ-HCH to cause diuresis among cockroaches, whereas organophosphorus compounds were less effective. And the data shown in Table 2 indicate that with combinations of custard apple seed extract with storage insecticides diuretic activity was enhanced as compared to the activity obtained with the application of the individual insecticide and custard apple seed extract.

Amounts of total haemolymph in the body of last instar nymphs of P. americana treated with individual and combination of insecticides: Amounts of total haemolymph in the body of last instar nymphs of cockroach was studied on the basis of the topical administration of the measured doses of the individual and mixture of insecticides separately on the 3rd abdominal sternum as described earlier and the data obtained are shown in Table 3.

Table 3. Amount of haemolymph in the body of last instar male nymphs of *P. americana* at different hours after the topical application of insecticide (alone or in combination).

Compound	LD_{50}* μg/g body weight		Amount of haemolymph (in ml/batch*) in the body of insects after treatment			
			Alone			Mixture
	Alone	Mixture	1 hr	3 hrs	18 hrs	18 hrs
Custard apple (Syn.)	1,500.00	—	1.11	0.9	0.65	—
γ-HCH	1.00	0.16	0.78	0.64	0.56	0.54
Malathion	12.50	2.00	1.40	0.52	0.50	0.48
Parathion	0.25	0.04	1.12	0.77	0.52	0.53
Control	—	—	—	—	1.52	

* Each batch consists of 3 individuals.

The data in Table 3 indicate that custard apple seed extract was more or less equal in effect to γ-HCH and other organophosphorus insecticides used to cause loss in haemolymph contents in the body of treated cockroaches. This effect increased with the increase in time, and it was maximum 18 hours after the application of insecticides. A similar observation was made with the combinations of custard apple seed extract and γ-HCH, malathion, fenitrothion and parathion.

Discussion

Water conservation is critical for insects because of their large surface area relative to volume and the generally arid environment in which they live. Their survival depends on the water proofing mechanism of the cuticle and the control of excretory and respiratory mechanisms which lead to water loss[12,13]. The present study indicates that the critical water balance is disrupted by poisoning with custard apple seed extract (a plant product), chlorinated and organophosphorus insecticides used, which is evident by the large amounts of rectal fluids measured in the rectum of treated insects. Paralytic doses used in the present study of the major types of compounds initiated massive excretion of fluid into the rectum of cockroach nymphs. It is therefore, concluded that the appearance of fluid in the recta of insects poisoned with insecticides is due to the presence in the haemolymph of a factor which induces rapid secretion of fluid by the malphigian tubules[8]. And, the rapid loss of a large volume of haemolymph by secretion of fluid into the rectum disrupts the critical water balance and the level of haemolymph in the body of the insect, which enhance the likelihood of the insect dying by dessication. Furthermore, the concentration of insecticide in the haemolymph increases as a result of the fluid loss and so enhance its disruptive action on the nervous system. Therefore, it is suggestive here that nonpersistent plant product in the environment could be used not as acutely lethal paralytic agents but as initiators of disadvantageous excretion.

The other points of interest are that custard apple seeds have manyfold advantage. 1) They have exhibited synergism with chlorinated and organophosphorus insecticides against cockroach nymphs. 2) They have shown toxic effect against cockroach nymphs. 3) They are used for curative purposes. 4) They have shown repellency aginst rice weevils. Therefore, it is further suggested here that the combinations of such material with insecticides may hold promise for the better control of insect pests with minimum health hazards.

Summary

Diuresis among last instar nymphs of *Periplaneta americana* treated with custard apple *Annona squamosa* seed extract was determined. Toxicological method used was topical application. This method indicated more accumulation of rectal fluid among last instar nymphs of *P. americana* treated with the measured doses of custard apple seed extract or γ-HCH as compared to malathion, fenitrothion and parathion.

The mixtures of custard apple seed extract and γ-HCH or malathion or fenitrothion or parathion exhibited synergism against last instar nymphs of *P. americana*. These mixtures enhanced diuretic activity as compared to individual treatment, and the level of body haemolymph was reduced with both the treatments which led to enhanced dessication among insects.

Acknowledgement: The authors wish to express their thanks to Dr. P. Ramchender Rao, Professor and Head, Department of Zoology, Post-Graduate Centre, Warangal-506001, A. P., India for his keen interest in the work.

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Further Studies of Aircraft Disinsection and Odor Characteristics of Aerosols Containing Resmethrin and *d-trans*-resmethrin[*1]. W. N. Sullivan[*2], A. N. Hewing[*2], M. S. Schechter[*2], J. U. McGuire[*3], R. M. Waters[*2], and E. S. Fields[*2] (Entomology Research Division, Agr. Res. Serv., US Department of Agriculture[*2], and Biometrical Services Staff, Agr. Res. Serv., US Department of Agriculture[*3], Beltsville, Md. 20705, USA.) Received November 12, 1974. *Botyu-Kagaku* 40, 5, 1975.

2. Resmethrin, *d-trans*-resmethrin 含有噴霧剤の航空機内における殺虫効果と残留臭気について[1]

W. N. Sullivan[2], A. N. Hewing[2], M. S. Schechter[2], J. U. McGuire[3], R. M. Waters[2], and E. S. Fields[2] (Entomology Research Division, Agr. Res. Serv., US Department of Agriculture[2], and Biometrical Services Staff, Agr. Res. Serv., US Department of Agriculture[3], Beltsville, Md. 20705, USA.) 49. 11. 12 受理

WHO から航空機内での殺虫剤噴霧の効力と使用後の乗客，乗員に与える影響について調査を依頼された．この目的に，resmethrin と *d-trans*-resmethrin の殺虫効果が評価されたが使用後に悪臭の残るという苦情があり，これを軽減するために，有効成分の純度の高いものを製造してもらい，さらにテストを行なった．

精製 resmethrin と90.2% *d-trans*-resmethrin を用いてジェット機内の閉塞空調条件下で殺虫テストを行なった．同様なテストは，長距離飛行中でも行ない，その製剤の使用で乗客が不快な匂いを感ずるかどうかを，噴霧前後にアンケートに回答してもらうことで調べた．石油系溶剤を含有しない噴射剤 P11 : P12 (1 : 1) だけの使用で，5 g/1000 ft^3 のスプレーが，イエバエ，カの駆除に効果があり，悪臭を残すこともなかった．

During 1971 the World Health Organization (WHO) conducted worldwide experiments with aerosols for the disinsection of aircraft at "blocks-away" (Sullivan *et al.*, 1972)[1]. The results indicated that an aerosol containing 2% resmethrin (NRDC-104; SBP-1382) in propellants 11 and 12 (50:50) without kerosene was effective and was accepted favorably by the passengers;an aerosol containing *d-trans*-resmethrin (bioresmethrin ; NRDC-107) was equally effective but was unfavorably received by the passengers. The subsequent investigation of the odors revealed that the residues from repeated application of either technical resmethrin or *d-trans*-resmethrin on glass plates exposed to direct sunshine for 1 to 3 days had an unpleasant musty or urine-like odor due to photodecomposition. A similar odor was produced in treated, closed rooms with sunshine filtering through windows and/or lighted with fluorescent tubes. The World Health Organization therefore requested the US Department of Agriculture to investigate the odor and possible means of reducing or eliminating it.

The manufacturers have made intensive efforts to refine their product and to add antioxidants to the mixture. The result was a technical product with a greatly improved odor as deter-

[*1] This paper reports the results of research only. Mention of a pesticide or a commercial product in this paper does not constitute a recommendation or an endorsement of this product by the US Department of Agriculture.